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NUTRITION REVIEWS

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ERRATA

The name of Dr. Charles Glen King, Scientific Director of The Nutrition Foundation, as Chairman should have been included in the list of members of the Scientific Advisory Committee to The Nutrition Foundation in the pages bound in the back of the December 1952 issue of NUTRITION REVIEWS.

Dr. King's name should also have been included in the list of the Board of Trustees in that issue, as a representative of the public, as should that of George A. Sloan, President of The Nutrition Foundation.

NUTRITION REVIEWS

VOL. 11 JUNE 1953 No. 6

THE CHALLENGE OF NUTRITION IN STATE HEALTH DEPARTMENT ACTIVITIES

Relationships of man to the food he eats have been known from time immemorial. However, it is only in the past century that this knowledge ceased to be empiric. During this time the science of nutrition demonstrated its intimate relationship to the growth, functions, maintenance and repair of living tissues. Nutrients were identified, and considerable information was accumulated about their functions and interrelationships. The study of food provided knowledge of the sources of these nutrients and their availability. It is ironic that this knowledge has not been put to more general use, since it supports the common experience of man in relation to food.

Effecting the wider use of nutritional knowledge is the challenge offered by nutrition to state health departments. This challenge is present in any health department program insofar as nutritional measures contribute to its objectives. These are the promotion of better health, the prevention of ill health, and therapy and rehabilitation from those conditions considered public health problems.

Because it is not possible to ignore completely man's need for food and what man is in relation to the food he eats, this challenge is being met to some extent in all state health departments. As a consequence, every department has nutrition activities, informal though some may be, and others unrecognized. It is from such beginnings that the concept of the role of nutrition in state health department activities may be evolved most easily.

A prenatal program of a bureau of maternal and child health will serve as an illustration. The physician who holds a prenatal clinic has an opportunity to learn something of the nutritional state of the patient through the medical history, physical examination and laboratory procedures. He gives general recommendations to the patient, with particular advice suited to her needs. Dietary instruction is included in some form. Promotion of optimum nutrition should be a routine part of prenatal care under the direction of the physician. Occasionally, the physician may have the nurse elaborate the general instruction on diet and include his particular recommendations. The physician responsible for the direction of other medical programs has a similar responsibility for nutrition, although it may not be so easily recognized nor so well accepted as in the above illustration. Maternal and child health, cancer control, tuberculosis control, dental health, crippled children's programs and chronic disease control are the programs which most readily come to mind.

Nutrition may enter any medical program through several approaches. The first concerns the nutritional characteristics of the group for whom the program operates, without regard for the specific condition which is the subject of the program. The second way has to do with the modifications of these characteristics which may be engendered by the condition itself. Another involves those in poor nutritional state as a result of other disease, stress, or faulty diet. An instance which includes these three approaches is the overweight child with residual paralysis from polio. He has the nutritional needs characteristic of his age,

which should receive primary consideration. These are modified by differences in activity, by the paralysis and by his overweight.

Administrative recognition of the potential role of nutrition in the several programs of the department is the essential first step. This can be obtained only through the administrator's close study of the contribution of nutrition to the effectiveness of his program, and through consultation with other persons qualified by training and experience to advise in such matters. This may be at a theoretic level. Only the administrator is in a position to have sufficient perspective to evaluate the unmet needs in his existing programs.

The second step involves the practical consideration by the administrator of the ways and means by which this nutritional knowledge can be used in the several programs. He needs detailed, specific information concerning the actual contribution this knowledge can make with reference to the objectives of the programs and to the people for whose benefit they operate. The medical director of a program is also responsible for the direction and operation of the nutrition services included. He must be sufficiently acquainted with the applied aspects of nutrition to direct the necessary in-service education as well as write and administer the program plan.

Experience has shown that the most effective way to include nutrition is through the services of the professional personnel who are already carrying out the program. The medical administrator can educate his staff as to the general principles of application, and call in assistance on details from persons more qualified to present this aspect. It is in this setting that the nutritionist can be used most efficiently and effectively, namely, by serving as a professional resource to all of the members of the health department. Public health nursing services are one of the most important means of reaching those individuals in groups who are not reached by other community activities. Justification for nutritionist positions in the department of health rests more securely on this concept of integration.

The realization of the importance of everyday services is frequently lessened by routine and by greater attention to more dramatic problems. Consultation is more likely to be called for on the latter. However, as integration progresses through staff education, the ordinary problems are recognized more readily and handled more adequately. This truly carries nutrition education into the life of the people.

This basic core of activity affords numerous other avenues for service. Consultation extends to other state departments. Occasional opportunities arise for joint staff education. As an example, budgeting for low income families is an apt topic for staff education not only of public health nurses, but of social caseworkers as well. This consultation also extends to other official and voluntary agencies and professional organizations which have responsibility for health and welfare of the population. All are concerned with long- and short-range implications in the greatest and widest use of the current knowledge of nutrition.

Nutrition thus becomes an integral part of the many programs in the health department, providing that essential continuity of service to professional people throughout the state, in contrast to the occasional response to sporadic requests from various local health departments and other agencies. A solid base is created on which the department staff is able to build, confident that nutrition consultation keeps them up-to-date and is available at predictable times. Every professional worker in the health department will be able to make practical application of nutritional knowledge in the routine course of his duties.

Although the emphasis is on meeting current problems, with seeming slight regard to the promotion of better health, what better way is there to work towards that goal than to solve these problems? The goal of better health is intangible, and progress

toward it hard to measure. However, this objective is not neglected. It is implied in all nutritional education, whether directed to the sick or the well.

The multitude of activities in which the nutritionist may be engaged derive their importance to the department insofar as they support the practical application of nutritional knowledge by the department staff in the course of their duties. There are usually a number of serious problems of which the department is aware, and an alert staff will discover others. It is the responsibility of the personnel of the department, in addition to the nutritionist, to establish the needs, determine the criteria for measurement, take preventive and corrective action, and provide the data for evaluation.

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IRON, HEMOGLOBIN AND ANEMIA IN INFANTS

Despite several gaps in our knowledge of iron metabolism in young infants, most of the factors concerned in normal hematopoiesis and in the development of nutritional anemias during the first year of life have become fairly well established. The iron content of the human fetus and neonate has been reinvestigated by E. M. Widdowson and C. M. Spray (*Arch. Dis. Childhood* **26,** *205 (1951)*). Analysis of total body iron of 13 immature fetuses and 6 stillborn infants weighing over 3000 g. showed a linear relationship between iron content and body weight.

This excellent study fails to support the hypothesis that most of the iron "stores" of the infant are acquired during the last trimester of fetal life. There was slight change in the concentration of iron in the body, differing little from that found in adults, 6 to 9 mg. per 100 g. of fat-free tissue. The average iron content of the 6 mature infants was 273 mg. Only 14 per cent of this amount was found in the liver and spleen. The majority of the iron present in the neonate is found in the circulating hemoglobin. These observations confirm an early study on this subject (M. L. Hugounenq, *J. de physiol. et de path. gén.* **1,** *703 (1899)*).

R. A. McCance and E. M. Widdowson (*J. Physiol.* **112,** *450 (1951)*) point out that the theory that the suckling obtains very little iron from his milk and has "stores" of this element in the liver came from observations by G. Bunge (*Z. f. physiol. chem.* **16,** *173 (1892)*) on the rabbit and guinea pig, the former having a liver "store" and a long suckling period and the latter negligible "stores" and a short suckling period. However, McCance and Widdowson demonstrated that the liver "stores" of the rabbit were not used for hemoglobin formation.

W. Lintzel, J. Rechenberger, and E. Schairer (*Z. f. d. ges. exp. Med.* **113,** *591 (1944)*) suggested that some species such as cats and the human are born with a great excess of circulating hemoglobin which constitutes the "store," while others, for example, rats, have negligible "stores" and must absorb iron freely from milk. A. St. G. Huggett and W. F. Widdas (*J. Physiol.* **110,** *386 (1950)*) confirmed these observations in the suckling rat using radioactive iron. McCance and Widdowson have shown that kittens, as well as rats, mice, guinea pigs and rabbits, obtain significant amounts of iron from milk. Human infants, with their relatively much slower growth rate, appear to be a special case, but existing studies on them of iron absorption from human or cow's milk are not so conclusive as the animal studies cited.

At birth the human infant has a plethora of circulating hemoglobin. The resulting physiologic fall in concentration can no

longer be attributed to increased rates of red cell destruction (C. A. Smith, "The Physiology of the Newborn Infant," *C. C. Thomas, Springfield, Ill. (1951)*). Smith has also compiled data from the literature concerning the changes in hemoglobin concentration following birth and the factors influencing these changes. It increases from about 17.0 g. per cent at birth to a slightly higher value the first day of life and then gradually falls to between 11 and 12 g. per cent, reaching this point after two or three months and remaining at this level throughout the first year of life. D. Gairdner, J. Marks, and J. D. Roscoe (*Arch. Dis. Childhood* **27,** *128, 214 (1952)*) have quantitated the hypothesis that these changes are the result of the response of the erythropoietic tissue to changes in the oxygen saturation of the arterial blood. A critical oxyhemoglobin concentration of about 11 g. per cent was found to explain these changes. In intrauterine life the oxygen saturation of the umbilical vein blood averages about 65 per cent or 11.4 g. of oxyhemoglobin when the total at birth is 17.6 g. The constancy of the umbilical vein oxygen content of the sheep fetus under varying oxygen tensions has been demonstrated by J. Barcroft ("Researches on Pre-natal Life," *Vol. I, C. C. Thomas, Springfield, Ill. (1947)*). A similar constancy of oxygen content in adult human beings living at various altitudes has been demonstrated (A. Hurtado, C. Merino, and E. Delgado, *Arch. Int. Med.* **75,** *284 (1945)*). A rapid increase in oxygen saturation to the adult level of 95 per cent occurs shortly after birth. The brief rise in the circulating hemoglobin concentration is thought to be a reflection of the momentum of the active erythropoiesis of fetal life.

That the gradual fall in hemoglobin concentration which ensues is due to decreased erythropoiesis alone is supported by quantitative studies of the bone marrow. Gairdner and co-workers made 102 examinations of the tibial marrow on 25 normal infants between birth and 3 months of age. Both total and differential counts of the nucleated cells were made. Both a relative and absolute decrease in red cells occurred, from 48.6 thousand per cubic millimeter on the first day of life to 2.8 thousand at 8 to 10 days of age. At about 2 months of age erythroid activity had increased (22.3 thousand cells). Similar marrow changes have been reported by K. Glaser, L. R. Limarzi, and H. G. Poncher (*Pediatrics* **6,** *789 (1950)*).

Samples of venous blood were taken at the time the marrow specimens were obtained, and hemoglobin and red cell concentrations, packed cell volume and reticulocyte percentages were determined. The total circulating hemoglobin was calculated using the blood volume data of P. L. Mollison, N. Veall, and M. Cutbush (*Arch. Dis. Childhood* **25,** *242 (1950)*), and the values obtained are only gross approximations. On the first day of life the total hemoglobin averaged 51.4 g. and contained 172 mg. of iron, equivalent to 63 per cent of the amount found by Spray and Widdowson. At 2 months of age the total hemoglobin had fallen to 31 g. and at 3 months had risen to 40 g. The reticulocyte percentage, which had fallen shortly after birth, was found to increase at 2 months of age, preceding the increase in total hemoglobin. Critical examination of the rate of red cell destruction in newborn infants suggested to Gairdner and co-workers that it did not take place at a rate greater than the adult normal and that the physiologic fall in hemoglobin concentration during the first two to three months of life was entirely due to suppression of erythropoiesis. Red cell production recommenced at about 2 months of age when the arterial oxyhemoglobin concentration again reached the critical level of 11 g. (95 per cent of the total hemoglobin).

In considering the iron economy of the human infant it is important to realize that

erythropoiesis is normally inactive during the first two or three months of life. As better methods of determining blood volume are applied to this problem it becomes more obvious that the infant at birth has supplies of iron in his circulating hemoglobin adequate for more than three months of growth. C. A. Smith *et al.* (*Am. J. Dis. Child.* **80,** *856 (1950)*) has demonstrated with radioactive iron that the transplacental iron in hemoglobin does not become diluted with exogenous iron until the age of 5 or 6 months.

Since studies of iron absorption using the tracer technic have not been performed on young infants we remain in ignorance concerning the availability of milk iron and its role in the iron economy during the first six months of the human suckling period. The evidence quoted above would suggest that the human infant may indeed be a special case among sucklings and be born with iron supplies adequate for almost the first half-year of life. Common experience attests to the fact that a diet of milk alone after that age is frequently associated with an iron deficiency anemia.

FAT-SOLUBLE SUBSTANCES IN MEASUREMENT OF BODY COMPOSITION

The high affinity of some anesthetics for fat suggests that body composition, specifically the amount of body fat, should be taken into account in prescribing the dosage of such anesthetics. B. B. Brodie *et al.* (*J. Pharmacol. Exp. Therap.* **98,** *85 (1950)*) have shown that thiopental belongs in this category. Earlier, B. de Boer (*Anesthesiology* **8,** *375 (1947)*) observed that semistarvation prolonged the sleeping time of dogs under thiopental anesthesia. G. Hermann and H. C. Wood, (*Proc. Soc. Exp. Biol. Med.* **80,** *318 (1952)*) undertook a study concerned with the influence of the fat content of the body on the course of thiopental anesthesia.

Starting with an initially homogeneous group of albino rats, by differential food allotments over thirty days the authors developed a "fat" and a "lean" group, with initial and final body weights of 175 and 199 g. ("fat") and 169 and 189 g. ("lean"). Both groups appeared active and healthy. The average fat content of the eviscerated rats, estimated from specific gravity following the method of E. N. Rathbun and N. Pace (*J. Biol. Chem.* **158,** *667 (1945)*), represented 13.5 and 8.5 per cent of the body weight.

In addition to thiopental (30 mg. per kilogram of body weight), injected into the tail vein, pentobarbital (25 mg. per kilogram of body weight) was used as control anesthetic, not characterized by a high affinity for fat. No animal received more than one anesthetic dose in forty-eight hours. With the duration of anesthesia measured from the time the righting reflex was lost to the time it was regained, the length of thiopental anesthesia was much shorter in the fat rats than in the lean animals (twenty-four versus fifty-three minutes). This difference may be legitimately ascribed to the existing differences in the fat content of the body since the duration of pentobarbital anesthesia was identical (twenty-seven minutes) in both groups.

What is the relative dosage of thiopental required by the two groups for a specified period of anesthesia? For twenty minutes the values are about 29 and 20 mg. per kilogram of body weight, for forty minutes about 35 and 24 mg. per kilogram of body weight in the fat and the lean animals, respectively. In percentage of the value for lean animals, the fat rats required about 45 per cent more. This would be explained if the 5 per cent of extra fat in the "fat" animals would take up eight times as much thiopental as the rest of the body. Such an

assumption is in reasonable accordance with the findings (Brodie *et al.*, *loc cit.*) that the adipose tissue of the dog takes up six to ten times as much thiopental as other parts of the body.

The study is a valuable contribution because it provides an empiric documentation for the idea that the dosage of some anesthetics should be adjusted with reference to the fat content of the body, rather than the gross body weight. The experimentation on man will demonstrate whether the effect of differences in body weight is of sufficient magnitude to be of practical importance. In view of the large variation in the fat content of the human body—an important facet of man's "nutritional status"—with the nonessential or "excess" fat ranging in clinically healthy men from 0 to 40 per cent of body weight (*cf.* E. F. Osserman, G. C. Pitts, W. C. Welham, and A. R. Behnke, *J. Applied Physiol.* **2,** *633 (1950)*), the consideration of this factor is likely to be practically significant.

The marked differences in the solubility of some substances in fat and the fat-free body mass can be utilized for developing methods for the estimation of the total body fat in the intact organism. Thus, the gaseous nitrogen is dissolved in body fluids and in body fat in a ratio of about 1 to 5 (9 cc. per kilogram of fluid, 52 cc. per kilogram of body fat). A. R. Behnke (*Harvey Lectures* **37,** *198 (1941/42)*; *Medicine* **24,** *359 (1945)*) has shown that it is possible to estimate the fat content from data on the volume of nitrogen removed from the body tissues by placing the body in an oxygen atmosphere.

The possibility of estimating body fat on the basis of the absorption of a nontoxic gas (cyclopropane), with a high fat:water solubility ratio, taken up by the animal organism in an enclosed system was examined by G. T. Lesser, A. G. Blumberg, and J. M. Steele (*Am. J. Physiol.* **169,** *545 (1952)*). The solubility ratio of cyclopropane in fat and the nonlipid tissues of the body is about 26:1. Under conditions of equilibrium about 90 per cent of the absorbed cyclopropane is present in the fat. This fact makes the cyclopropane method relatively independent of the proportion of the nonlipid substances contained in the body. The total amount of fat, in grams, is calculated by dividing the solubility coefficient of cyclopropane for rat fat at body temperature (milliliters per gram of fat) into the milliliters of cyclopropane absorbed by fat.

The fat content of 10 white rats was determined *in vivo* from measurements of cyclopropane absorption. For the purposes of direct chemical analysis of the fat content the animals were killed by rapid addition of cyclopropane to the chamber at the end of the period of observation. The fat content of the whole body was determined by extraction using ethyl ether as solvent. The weights of the animals varied from 236 to 373 g. (mean 303 g.), the total body fat from 25.0 to 70.4 g. (mean 40.1 g., or 13.2 per cent of the body weight).

The mean value of fat obtained by the cyclopropane absorption method (40.1 g.) was very close to that obtained by ether extraction. For individual animals the differences, expressed as percentages of the ether extract values, ranged from −12 to +9 per cent. These differences decrease considerably when fat is calculated not in grams but as percentage of body weight. While the technic is complicated, the method is promising and may be particularly useful as a check on some basic controversial points raised by the estimation of body fat from specific gravity and from the total body water. The principal source of difficulty, needing critical analysis and clarification, is the fact that the specific gravity method uses the concept of *lean body mass,* containing the "essential lipids," as its point of departure, while the estimates of fat from total body water start with the *fat-free* body weight, in the strict chemical sense. This confusion is exemplified by T. C. Prentice *et al.* (*J. Clin. Invest.* **31,** *412 (1952)*) who

speak of "the fat-free portion (of the body), called the lean body mass." However, in reference to this point, they are in a large and distinguished company.

FLUORIDE EXCRETION IN MAN

The increasing use of fluorides as prophylactic agents for dental caries has been accompanied by a number of excellent clinical and laboratory studies. These have dealt with such varied aspects of fluoride use as the effectiveness of complex compounds of fluorine, the relationship of malnutrition to fluoride toxicity, and the effect of fluoride upon dental caries in adults (*Nutrition Reviews* **10,** *74, 114, 227 (1952)*). For an agent which may well see increasingly widespread use, however, there have been relatively few well controlled studies of its excretion in human subjects (F. J. McClure, H. H. Mitchell, T. S. Hamilton, and C. A. Kinser, *J. Indust. Hyg. Toxicol.* **27,** *159 (1945)*; McClure and Kinser, *Pub. Health Reports* **59,** *1575 (1944)*).

E. J. Largent (*Arch. Indust. Hyg. Occup. Med.* **6,** *37 (1952)*) has investigated the elimination of fluoride in the urine in 4 individuals whose fluoride intake was known throughout the period of study. Three of these persons (2 males and one female) had previously lived in low-fluoride drinking water areas and just previous to the excretion study had ingested large amounts of fluoride (3 to 36 mg. daily) over two- to one hundred and thirty-week periods. The fourth subject was a native of Amarillo, Texas, and had been exposed for twenty years to the high fluoride content (2.4 to 4.4 parts per million (p.p.m.)) of the water in that community, but had not participated in the previous experimental ingestion of fluorides. All subjects were followed for nineteen days during which they received a diet and water yielding a low fluoride intake (0.4 to 0.8 mg. daily). Diet and water ingested during this period were analyzed for fluoride as were urine and fecal collections.

In the 3 persons whose intakes were known previous to this period the probable storage which took place during the high intake period was estimated at 500, 1700, and 1900 mg., respectively. These values were determined by subtraction of the amounts of fluoride excreted during the experiment from the amounts ingested. Storage in the resident of Amarillo was, however, not known. During the nineteen-day low intake period the first 3 patients excreted 8.04, 13.7, and 36.1 mg., respectively, in excess of the fluoride intake while the Amarillo native eliminated 25.7 mg. Since the amount excreted by the 3 experimental subjects appeared to be related to the amounts previously stored, the author assumed that the apparent storage in the latter individual was about the same as for the second and third subjects, or 1700 to 1900 mg. This then would represent the approximate storage of a person living for twenty years in an area containing 2.4 to 4.4 p.p.m. fluoride in the drinking water. As such, this would indicate that some fluoride storage occurs at this intake level, a fact which is not unexpected and which is not contrary to other literature in this field. Indeed, this result supports the suggestion of McClure and co-workers (*loc. cit.*) that fluoride ingested in water with concentrations of less than 4 to 5 p.p.m. is eliminated in excess of 90 per cent in the urine. For the sake of discussion, let us make the reasonable assumptions that the Texan in this study consumed an average of one quart of water per day and that the average fluoride content was 3.5 p.p.m. In the course of twenty years, he would have consumed a total of about 30 g. of fluoride, of which 1.7 to 1.9 g. has been stored. Of additional interest in this study

is the fact that radiologic analysis revealed no evidence of fluoride in the bones of this Texas native, nor in the other subjects.

Evidence suggesting that daily ingestion of 3 mg. of fluoride may lead to significant storage is also available from one of the known intake subjects. This subject took 3 mg. of fluoride as sodium fluoride daily for forty-five weeks and attained a storage of 500 mg. during this period. This is not in keeping with the results obtained previously by McClure and his group, and represents a storage of about 53 per cent. The question arises here as to whether such a high storage rate may have occurred during this reasonably short period, but would have plateaued at a lower level if the experimental period had been appreciably longer.

Additional studies of the 3 subjects who were given known amounts of extra fluoride were carried out over periods of twenty to ninety-seven weeks of low fluoride intake. Curves of excretion were constructed from a formula obtained from the data, and from the curves it was possible to determine the time necessary for these subjects on low fluoride intake to reach base excretion levels at which intake would not be exceeded by output. These figures ranged from two to four and seven-tenths years for these individuals.

A limited analogy may be drawn from excretion studies carried out by G. H. Collings, R. B. L. Fleming, R. May, and W. O. Bianconi (*Arch. Indust. Hyg. Occup. Med.* **6,** *368 (1952)*), who studied acute fluoride excretion in subjects occupationally exposed to fluoride-containing atmospheres. Although actual intakes could not be measured, excretion rates in a worker habitually exposed to this atmosphere were higher than those of a control subject exposed to the same atmosphere on a single occasion. Similar curves of elimination were obtained after the exposure was ended, but the exposed worker's excretion rate was consistently higher than that of the control. Urinary excretion by a group of other workers also showed marked elevation after an exposure period at work and high urinary excretion continued just prior to the next work period twenty-four or forty-eight hours later. Such elevated excretion is presumed to be due to fluorides previously stored in the tissues of these workers. The various excretion levels reached by these men reflected the different levels of fluoride in the work atmospheres to which they were exposed.

While these investigations of fluoride elimination are based upon only a few individuals, they suggest that storage may occur at previously unsuspected intake levels. As a measure of accumulated storage, fluoride balance may be considerably more crude than is intimated in the report by Largent, but the technic of urinary excretion is unquestionably of value as an indicator of whether an individual has received sufficient exposure to fluorides to build up stores of this element.

This type of study has not as yet been applied to groups of individuals simultaneously assayed for the incidence of caries, but further exploration of this possibility might enlarge the body of information available on fluoride metabolism.

There seems to be little likelihood that toxic amounts of fluoride could be accidentally ingested as a result of an artificial water fluoridation program. However, there are extremely few good data concerning the total subject of fluoride metabolism. If ample data were available to establish conclusively that the amount of fluoride ingested by continual use of water containing 1 p.p.m. of fluorides was innocuous even though 2 to 3 g. might be stored in the skeleton in the course of a lifetime, then it would be relatively easy to answer the numerous critics of artificial fluoridation who are causing postponement of this program. On the other hand, if this amount of fluoride ingestion is harmful, then this fact should be known as soon as possible. On the basis

of a conservative analysis of all available facts concerning fluorides, there seems to be no evidence whatever to suggest that the presently proposed levels of water fluoridation can have anything other than decidedly beneficial results. However, such a hypothesis should not serve as an opiate to stifle fundamental studies of fluoride metabolism, or further investigations on the causes and control of tooth decay.

THE WATER REQUIREMENT OF INFANTS FED CONCENTRATED FORMULAS

As has been pointed out (*Nutrition Reviews* **8,** *1* (*1950*)), water occupies the "number 1 position" among the essential nutrients since the human organism succumbs to a deficiency of this substance in only a few days. Water is of particular importance to the premature and young infant for the excretion of the electrolytes present in the diet because of the relatively poor ability of these subjects to concentrate their urine. Consequently, provision of an adequate intake of water in relation to the solute concentration of the formula becomes necessary. Adequate amounts of water are provided to infants fed diluted evaporated or whole milk formulas with the addition of carbohydrate to maintain the caloric concentration at 0.67 calories per milliliter. Since unmodified cow's milk could be expected to impose an added solute load on the kidney, E. L. Pratt and S. E. Snyderman (*Pediatrics* **11,** *65* (*1953*)) have studied the renal water requirement of 6 premature infants fed isocaloric diets containing two different solute loads.

For the purposes of this study hypercaloric formulas were used which contained 0.9 calories per milliliter. The "unmodified" mixture consisted of 100 ml. of evaporated milk diluted with water to a final volume of 155 ml. The "modified" mixture contained 100 ml. of evaporated milk, 14.2 g. of dextrimaltose, and water to a final volume of 220 ml. These isocaloric formulas were consumed in similar amounts. One half of the infants received the "unmodified" formula for six days, followed by the "modified" formula for a subsequent six-day period. The reverse order was used for the other 3 infants. Two twenty-four hour urine specimens were collected at the end of the experimental periods. The total volume and freezing point depression of each sample were measured. The osmolarity of these specimens was calculated and the daily renal solute load determined. It was presumed that the extrarenal water losses by these infants were constant during the period of this study.

The "modified" evaporated milk formula provided an average of 19.7 milliosmols per kilogram of body weight per day to the kidney for excretion. The load imposed by the "unmodified" formula was 85 per cent greater (36.4 milliosmols per kilogram of body weight per day). Statistically this difference was highly significant. When these results are expressed in terms of renal water requirement and supposing that the concentrating power of the kidney was maximal, the values would be 28 and 52 ml. per kilogram per day respectively. Although this amount of water is readily available to normal infants, any limitation in the amount of water available to the body for this purpose would overtax the limited concentrating power of the immature kidney and retention of electrolytes would ensue. The loss of large amounts of water by extrarenal routes occurs both in diarrhea (L. E. Holt, A. M. Courtney, and H. L. Fales, *Am. J. Dis. Child.* **9,** *213* (*1915*)) and subsequent to hot weather (*Nutrition Reviews* **10,** *293* (*1952*)). When the subject is faced by these losses even in their milder clinical manifestations the need for adequate

water for the renal excretion of the dietary solutes not needed by the tissues of the body becomes extremely important.

Although the experiments of Pratt and Snyderman were performed using formulas more concentrated that those customarily used in infant feeding, their emphasis upon the role of added carbohydrate in the indirect provision of water for renal solute excretion is pertinent. The use of dilute evaporated milk mixtures incorporating added carbohydrate to make them isocaloric with human milk has been eminently successful in the feeding of small infants. The success of such formulas in meeting the nutritional needs of infants may be due to factors that are not readily appreciated. Pratt and Snyderman noted the present-day trend toward the simplification of formulas and particularly the tendency toward omission of supplementary carbohydrate. Since the protein content is increased by this means, the concomitant increase in renal solute load may be overlooked by the physician wishing to provide this nutrient in ample amounts. The infant subjected to unusual extrarenal water losses will then have a smaller margin of safety in his overall water balance.

BLOOD GLYCINE IN ARTHRITIS

Some interesting observations on the response of the blood glycine concentration to the administration of benzoic acid have been made by H. M. Lemon, W. H. Chasen, and J. M. Looney (*J. Clin. Invest.* **31,** *993 (1952)*). These investigators studied 54 patients with rheumatoid arthritis in various stages of activity and 41 control patients, most of whom were suffering from other forms of arthritis or from metastatic cancer. The concentration of glycine in the blood was determined chemically before and at thirty-minute intervals for two hours after the injection of 1.77 g. of sodium benzoate (modified after Quick's hippuric acid test).

It was found that most of the serum glycine of the nonrheumatoid individuals after benzoate administration remained unchanged or declined less than 15 per cent one hour after the injection. In contrast, 35 of the 54 patients with rheumatoid arthritis showed a decline of greater than 15 per cent of the initial level an hour after the injection of sodium benzoate. By suitable statistical analysis the authors concluded that there was a highly significant difference between the group of patients with rheumatoid arthritis and those without this disease insofar as their ability to maintain the serum glycine concentration after the injection of sodium benzoate was concerned. The test is, of course, not a specific one since there was considerable overlapping of the two groups. Nevertheless, the finding of a positive test in a specific patient, the authors believe, may aid in the early diagnosis of rheumatoid arthritis since patients in the first stage of this disease (without radiologic signs) had positive tests about as frequently as those with the more severe disease. There did not seem to be an increase in the positiveness of the test with increasing severity of the rheumatoid arthritis.

The authors discuss possible mechanisms for this failure to maintain serum glycine levels and suggest that there may be decreased reservoirs of glycine, decreased synthesis, or an increased utilization of glycine. They note that glycine is a very important part of collagen, which is increased around the joints in most of these patients. They also point out the possibility that diminished supply of glycine might impair the production of normal collagen. Finally, the authors suggest that the increased hippurate excretion in anxiety states or following severe stress might lower

the available glycine pool. The fact that anxiety and stress are predisposing factors in rheumatoid arthritis might bear an etiologic relationship by removing glycine.

It would seem reasonable to conclude that many patients with rheumatoid arthritis are not able to maintain normal serum glycine levels after the administration of sodium benzoate as well as do nonrheumatoid patients. Just what the relationship between this finding and the disease rheumatoid arthritis is, especially with regard to collagen formation, remains for future investigation.

LOW FAT DIET IN TREATMENT OF MULTIPLE SCLEROSIS

Multiple sclerosis is a disease of the nervous system associated with demyelinization in focal areas of the brain, brain stem and spinal cord. Although a variety of factors have been proposed as having roles in the causation of this disease, the etiology remains obscure. The distribution of multiple sclerosis among different population groups has been studied by a number of investigators in the effort to determine the conditions which favor occurrence of the disease. The epidemiologic findings have been reviewed by L. T. Kurland (*Am. J. Med.* **12,** *561 (1952)*). The most consistent observation of this type has been the relatively greater incidence in northern latitudes and in colder climates.

One series of epidemiologic investigations indicates that the character of the diet may condition the occurrence of multiple sclerosis (*Nutrition Reviews* **10,** *256 (1952)*). R. L. Swank (*Am. J. Med. Sci.* **220,** *421 (1950)*) compared the incidence of the disease with estimated dietary fat intake in Norway, Denmark, Holland and Switzerland. In Norway and Switzerland there was apparent correlation of increased incidence of multiple sclerosis with periods and areas of highest fat consumption. Later observations (Swank, O. Lerstad, A. Strøm, and J. Backer, *New Engl. J. Med.* **246,** *721 (1952)*) indicated that incidence was highest in areas where farming and milk production were the chief industries, and where diets were consumed which were not only high in total content of fat, but also contained large quantities of milk and animal fat. These investigators concluded that their findings suggest but do not prove that ingestion of relatively large quantities of fat may be one of the factors which induce multiple sclerosis in susceptible individuals. They are careful to state, however, that factors other than dietary fat may have similar geographic distribution and may, thereby, be of equal or greater significance in the occurrence of multiple sclerosis.

Since epidemiologic studies and dietary surveys indicated a possible role of a high fat diet in the etiology of multiple sclerosis, it seemed reasonable to determine the effect of dietary restriction of fat on the course of the established disease. A report concerning the effect of low fat diets on the clinical course of multiple sclerosis has appeared. The observations are those of the same investigator, whose studies of the epidemiology of the disease are referred to above (R. L. Swank, *Arch. Neurol. Psychiat.* **69,** *91 (1953)*). Forty-seven patients with clinically established multiple sclerosis have been provided a low fat diet for periods of from two and one-half to four years. These patients have also been treated with various supportive measures, drugs to relieve nervous tension, and other forms of therapy as indicated.

The diet utilized provides less than 40 g. of fat daily. There has been some manipulation of the fat portions of the diet during the four years of the study. In the last year, milk fat has been excluded from the diet and replaced by animal fat or other hard fats, such as margarine and shortening. The

daily diet provides 50 or more g. of protein, depending upon the body weight of the patient, and carbohydrate sufficient to meet the balance of caloric needs. A capsule providing a daily supplement of vitamins A, D, C and B-complex is given also. Each patient records the quantity of all foods consumed. These records are evaluated by a dietitian at two weekly or monthly intervals, so that the diet may be adequately controlled.

The results of this form of dietary therapy might have been more adequately evaluated had a group of similarly selected patients been followed for the same length of time while consuming a diet without restriction of fat. However, a sufficiently large group of patients to effect this control was not available. The investigator attempted an evaluation of the therapeutic effect of the low fat diet by comparing the course of the disease in each individual before and after the institution of this dietary regimen. Multiple sclerosis is characterized by a course of varied progression and severity and by episodes of remission and exacerbation. The patient's history and recollection of the prior course of his disease and the data from hospital charts were utilized in describing the course of illness prior to institution of treatment by diet. The patients were seen at frequent intervals and all changes in symptoms and physical signs were carefully recorded during the period on the low fat diet. It must be assumed that the clinical information obtained during periods of treatment was more adequate and detailed than that available for the pretreatment periods.

The author divided the patients into groups according to the nature and course of the disease in the attempt to determine the effectiveness of treatment. Thirty-four of the patients had forms of multiple sclerosis which were characterized clinically as being early in the course but associated with previous exacerbations and remissions. The performance category which represented an evaluation of the nature and extent of neurologic lesions was determined before and during the course of treatment. The patients consumed the low fat diet for periods ranging from two to four years. During this time clinical evaluation indicated slight to pronounced improvement in 25, slight deterioration in 3, and no change in 6 patients. Exacerbations of the disease occurred at average intervals of ten months in the pretreatment period. These periods of disease activation were not eliminated by consumption of the low fat diet in these patients. However, the average interval between attacks was lengthened to thirty months. Certain of these exacerbations followed dietary indiscretions in which the fat intake was greatly increased. If such attacks were eliminated from consideration, the mean interval between exacerbations was further lengthened to forty-two months. Moreover, the majority of such attacks occurred within the first year of treatment, indicating a greater effect with more prolonged ingestion of the low fat diet. The clinical evaluations of periods of disease activity indicated that exacerbations were shorter and less severe while patients were receiving dietary treatment.

In a group of 13 patients with late or progressive forms of multiple sclerosis, there was no indication of improvement in physical performance during an average period of three years of treatment with the low fat diet. However, exacerbations of the disease which occurred were milder and the average interval between attacks was lengthened from eighteen months in the pretreatment period to sixty-six months.

It is concluded that the low fat diet used in this study of the treatment of multiple sclerosis was of considerable effectiveness. No claim for the cure of the disease is made. However, the author was impressed with observations that are not clearly apparent in his data. These relate to general clinical improvement in his patients and their increased capacity to work and make a living, despite the occurrence of exacerbations of mild form. The point is made that

if this form of therapy is to modify the course of multiple sclerosis significantly, it must be started early, or soon after the diagnosis is achieved.

A number of considerations are offered which might relate high fat intake to the occurrence of multiple sclerosis. One concept of the etiology of the disease relates to the production of local areas of ischemia in the central nervous system. It is pointed out that this might result from the adhesive clumping of red blood cells (sludging) noted after large fat meals in dogs and human subjects (R. L. Swank, *Am. J. Physiol.* **164,** *798 (1951)*). However, no evidence has been obtained of altered suspension stability of the blood of patients with multiple sclerosis. Other experimental evidence is cited indicating that feeding of fat is followed by a decrease in the number of circulating blood platelets and a shortening of the clotting time of the blood. Whether these factors pertain in multiple sclerosis and are important in the production of vascular lesions in the central nervous system is not known.

The observations described by Swank would seem to indicate a possible benefit from the treatment of multiple sclerosis with a low fat diet. The author's enthusiasm for this form of therapy is apparent despite his efforts at caution in interpretation of his data. Much remains to be done before a beneficial effect from a low fat diet may be accepted without qualification. Further clinical observations and controls are indeed required. The interpretation of the effects of dietary management on the course of any disease in the human being must be approached conservatively and with reservation. The difficulties are enhanced in a widely disseminated disease of the nervous system, such as multiple sclerosis. In these diseases, clinical manifestations may be of great variety and the course subject to frequent and unpredicted exacerbations and remissions. If the author's concept of a role of the high fat diet in producing multiple sclerosis is correct, it is conceivable that a similar disease might be produced in the experimental animal. Certainly, investigative efforts of this type should be undertaken.

BOW LEGS IN DUCKS IN NIACIN DEFICIENCY

In studying the vitamin D requirement of ducklings, J. C. Fritz, W. Archer, and D. Barker (*Poultry Sci.* **20,** *151 (1941)*) reported the occurrence of bowed legs, a condition which was also observed in ducklings by I. Motzok and H. D. Branion (*Ibid.* **27,** *482 (1948)*) under somewhat similar experimental conditions. Bowed legs have been reported to occur in ducklings fed practical rations (G. F. Heuser, M. L. Scott, R. K. Eskew, and P. W. Edwards, *Ibid.* **30,** *672 (1951)*). In a preliminary study, Scott and Heuser (*Ibid.* **31,** *752 (1952)*) reported that niacin deficiency was responsible for the bowed-leg condition. A detailed report of the influence of niacin and natural sources of niacin (yeast or wheat) on this condition in ducklings was recently reported by Heuser and Scott (*Ibid.* **32,** *137 (1953)*).

Pekin ducklings (20 to 25 per treatment) were used for the study. They were housed in heated pens with wire mesh floors. The basal ration used had the following per cent composition: corn meal, 40; wheat flour middlings, 15; wheat standard middlings, 15; pulverized oats, 10; fish meal, 8; soybean oil meal, 10; dicalcium phosphate, 1; limestone, 0.5; salt (iodized), 0.25; D-activated animal sterol, 0.1; and stabilized dry vitamin A, 0.1. Riboflavin supplement and manganese sulfate each were added at the rate of 12 g. per 100 pounds of ration. In preliminary trials it had been established that high levels of cod liver oil, manganese,

or choline were ineffective in preventing the bowed legs.

When the basal ration alone was fed, all the ducklings developed the bowed-leg condition by the end of a three-week feeding period. When 2 per cent of fish liver oil was added to the basal ration, the ducklings likewise developed the bowed-leg condition. Dried skim milk, dried whey, grass juice, vitamin B_{12}, or aureomycin were likewise ineffective when added to the basal diet with fish liver oil. Supplementing the basal ration with 5 per cent of dried brewers' yeast resulted in almost complete protection against the bowed legs, while a similar level of dried distillers' solubles gave partial protection.

Further studies indicated that a supplement of 2.5 per cent of brewers' yeast reduced the incidence of bowed legs to 28 per cent, while a supplement of all known vitamins reduced the incidence of bowed legs to 8 per cent. The omission of vitamins from this mixture, one at a time, revealed that the bowed-leg condition was apparent only when niacin was omitted. Heuser and Scott further noted that the niacin supplement required to prevent the bowed-leg condition was somewhere between 5 and 10 mg. per pound of ration. The former level resulted in a 45 per cent incidence of the condition, whereas the latter level gave complete protection. Comparison of crystalline niacin with niacin in yeast revealed that they were equally effective in preventing the bowed legs. On the other hand, a supplement of 28 per cent of standard wheat middlings, containing 5 mg. of niacin as determined microbiologically, was completely ineffective.

Niacin supplement to the basal ration not only completely prevented the bowed-leg condition, but also generally improved the growth rate of the ducklings. Ducklings receiving niacin or natural preventive substances usually weighed 200 to 300 g. more than the control animals, the weight of which usually equalled about 1000 g. at the end of a three-week experimental period. Antibiotics (aureomycin, bacitracin, penicillin) were tried in the basal ration in a number of trials and were found to have no appreciable effect on growth and had no preventive or curative value for the bowed-leg condition. The 2 per cent fish oil supplement invariably produced a growth depression that could not be overcome with any of the supplements tested.

The bowed-leg condition apparently is not related to the enlarged hock disorder that has been noted in turkeys fed practical rations, since the latter condition responded to grass juice (*Nutrition Reviews* **10,** *111 (1952)*). Both conditions are readily preventable when yeast is included in the diet.

The bowed-leg condition in varying severity was reported by the authors to occur in commercial flocks of ducklings. This represents serious losses, since the affected animals usually do not make normal weight gains, and severely affected animals cannot be marketed readily.

The basal ration contained 13.6 mg. of niacin per pound, as determined by microbiologic assay. Eight mg. of niacin per pound of ration is considered adequate for growing chickens. The results of Heuser and Scott thus indicate that the niacin in the basal ration is largely unavailable for ducklings, or that the duck has a relatively higher niacin requirement than other avian species.

NUTRITIONAL VALUE OF CORN PROTEINS

The Illinois Agricultural Experiment Station, starting with a variety of corn which contained 10.92 per cent of protein, has produced two corn strains with markedly dif-

ferent protein contents. One of these, the high-protein strain, after 50 generations of selection now contains an average of 19.45 per cent of protein, while the other, the low-protein strain, now averages 4.91 per cent of protein (C. M. Woodworth, E. R. Leng, and R. W. Jugenheimer, *Agronomy J.* **44,** *60 (1952)*).

Although all the major parts of the corn kernel contribute, though in different degrees, to the increase in protein in high protein varieties, the horny endosperm apparently increases more than the starchy endosperm. Since 60 to 65 per cent of the zein in corn is in the endosperm, this protein increases more rapidly than any of the others as the protein of the corn is increased. This increase may be accomplished through selective breeding, or through soil treatment (level of soil fertility, particularly with respect to nitrogen) (T. S. Hamilton, B. C. Hamilton, B. C. Johnson, and H. H. Mitchell, *Cereal Chem.* **28,** *163 (1951)*).

In a recent paper from the Illinois laboratory, it was reported that an increase of 1 per cent in the total protein of corn will increase the proportion of zein by about 5 percentage units on the average. This was true for corn varieties ranging from 6 to 14 per cent in protein content (H. H. Mitchell, T. S. Hamilton, and J. R. Beadles, *J. Nutrition* **48,** *461 (1952)*). These investigators studied the digestibility and the biologic utilization of absorbed corn protein for maintenance and growth in rats.

Corn varieties and soil treatments were selected such that total protein contents of the corn varied from 7.3 per cent to 21.0 per cent. The data clearly revealed the effects of the proportionally greater rise in zein compared to other proteins with respect to the amino acids that are limiting in zein.

The tryptophan in the total protein averaged 0.55 per cent in corn containing 20 to 21 per cent of protein, whereas this amino acid averaged 0.87 of the total protein in a corn variety containing 7.3 per cent of protein.

With respect to lysine, this amino acid averaged 1.8 per cent of the total protein in the high-protein varieties, contrasted to 2.9 in the low-protein variety. For both these amino acids, values intermediate between these extremes were found in the corn containing 10 or 13 per cent of total protein.

The nutritional studies with rats indicated that the average digestibility of the corn protein increased as the total protein of the corn increased. In general, for each 1 per cent increase in the total protein content of corn there was an increase of about 0.4 per cent in digestibility of the protein, the high-protein corn averaging 95 per cent in digestibility of protein and the low-protein corn averaging 88 per cent. With respect to biologic utilization of the protein (percentage of absorbed corn protein that was retained for purposes of growth or maintenance), the low-protein corn was clearly better than the high-protein corn. The protein in the former averaged 69 per cent in biologic value, while the latter averaged 45 per cent.

It should be emphasized that the low biologic value of the proteins of corn, and the decreasing biologic value as the protein content increases, is not a serious problem insofar as animal feeding is concerned. The Illinois workers demonstrated that supplementation of the corn protein with the amino acids tryptophan and lysine increased the biologic value of both low- and high-protein corns. Their results further indicated that the protein of high-protein corn was equal in biologic value to that of low-protein corn when it was fed to growing rats in a diet containing a mixture of meat scraps, soybean oil meal, and alfalfa meal, a protein-containing adjunct generally used to supplement the proteins of corn in poultry and swine rations. In this feeding trial, the ration was prepared to contain approximately 18 per cent of protein, and only 12 per cent of the supplement was needed to bring the protein content of the ration to this level when high-protein corn was used,

contrasted with a level of 26.5 per cent of the supplement required to bring the protein content to the 18 per cent level in the low-protein corn ration. This obviously means that the value of increased protein in high-protein corn under practical conditions of mixed protein feeding more than offsets the apparently lower nutritive value (lower biologic value) of the protein of such corn when fed alone. The Illinois workers therefore conclude that high-protein corns can be effectively supplemented in swine feeds, and probably also in poultry feeds, and that high protein corns will materially decrease the level of protein supplements needed in such feeds.

Another investigation concerned with the nutritional value of corn is reported by workers in Mexico (R. O. Cravioto, G. H. Massieu, O. Y. Cravioto, and F. de M. Figueroa, *J. Nutrition* **48,** *453* (*1952*)). These workers studied the effect of untreated corn and Mexican tortilla upon the growth of weanling rats fed diets low in niacin and tryptophan, with and without supplements of cystine. A basal diet was used which was similar to those used by many other investigators who have studied the influence of corn supplementation on a niacin-low diet. The basal mixture contained casein (11.66 per cent), a salt mixture, and sucrose, and was fed by mixing 60 parts of this basal mixture with 40 parts of the corn or tortilla.

Corn was noted to inhibit growth when the niacin-low diet contained a cystine supplement, but tortilla actually stimulated growth on the cystine-supplemented diet. Supplementation with niacin corrected the growth-depressing effect of corn, whereas this supplement was of no value on the tortilla diet. The growth-depressing effects of corn could not be obtained when the cystine supplement in the basal mixture was omitted. This is in harmony with the results of others who have demonstrated that the sulfur-amino acid deficiency of low-casein diets must be corrected in order to demonstrate the tryptophan deficiency produced by feeding corn on such diets (*Nutrition Reviews* **10,** *135* (*1952*)).

The results reported by the Mexican workers are in agreement with those reported by J. Laguna and K. J. Carpenter (*see Nutrition Reviews* **10,** *105* (*1952*)), who noted that the growth-depressing effects of corn, when added to low-casein diets, could be eliminated by treating the corn for forty minutes with aqueous calcium oxide, the so-called wet-milling process. These workers explained their results on the basis that the lime treatment liberated niacin precursors which are normally unavailable. Such an explanation, however, does not appear to satisfy the recent results of Cravioto and associates (*loc. cit.*), since they found no difference in the niacin content of corn and tortilla as measured by microbiologic assay. An alternate explanation offered by the Mexican workers was that certain amino acids in corn may be destroyed or racemized by the alkali treatment and that this might correct other amino acid imbalances of low-casein diets. The amino acids most likely to be thus affected are arginine, threonine and histidine, and the former two have been shown to be involved in producing imbalance on a casein-gelatin diet (*Ibid.* **10,** *135* (*1952*)).

The results of Cravioto and associates and those of Laguna and Carpenter show clearly that lime-treated corn in some way alleviates the tryptophan-niacin deficiency when it is substituted for raw corn in diets low in these dietary essentials. Whatever may be the explanation of this effect, it may be the reason for low pellagra incidence in certain corn-eating populations such as that of Mexico.

The Illinois studies emphasize again that vegetable protein deficiencies with respect to essential amino acids are not a problem if such proteins are fed in well-balanced diets containing several plant and animal protein sources.

EFFECT OF CALCIUM CARBONATE IN THE DIET

Several reports by workers at the Rowett Research Institute in Great Britain have dealt with deleterious effects of calcium carbonate additions to the diet. A pyridoxine deficiency induced in suckling rats by excess thiamine was accentuated, and the growth of weanling rats was retarded by calcium carbonate additions to the diet (M. B. Richards, *Brit. J. Nutrition* **3,** *109* (*1949*)). In adult rats, calcium carbonate additions to the diet resulted in increased iodine excretion and diminished iodine concentration in the thyroid gland (B. W. Simpson, *Biochem. J.* **41,** *xlv* (*1947*)).

J. W. Howie and G. Porter (*Brit. J. Nutrition* **4,** *175* (*1950*)) noted that a slightly modified Sherman B diet composed of 66 per cent of ground whole wheat, 33 per cent of dried whole milk, and 1 per cent of sodium chloride, gave better reproductive results in mice than the same diet in which 6 per cent of the wheat was replaced by 5 per cent of casein and 1 per cent of calcium carbonate. Recently, M. B. Richards and W. A. Greig (*Ibid.* **6,** *265* (*1952*)) have extended these studies.

Four basal diets were used. The first was identical with that used by Howie and Porter. The second and third were the same except that 6 per cent of the wheat was replaced by an equal amount of cornstarch and casein, respectively. The fourth basal diet was similar to the Howie and Porter diet except that it contained a supplement of 5 g. of casein for each 100 g. of the basal mixture.

The experimental plan was a factorial design in which four levels of calcium carbonate (0, 0.5, 1.0, and 2.0 per cent) were tried as supplements to each of the four diets. Four female mice were assigned to each of the 16 experimental treatments and the objective was to determine the influence of the calcium carbonate on breeding performance.

There were no significant differences that could be attributed to the basal diets, but there were striking effects attributable to dietary level of calcium carbonate. The 16 females receiving the basal diets with no added calcium carbonate had 125 young born in first litters, 82 of which were weaned and 43 of which died before weaning age. The 15 females that bore first litters on the diets containing 2 per cent of added calcium carbonate produced 105 young, 39 of which were weaned and 66 of which died before weaning age. This represented a survival of 65 per cent at weaning for young from mothers receiving the basal diets only, and a survival of only 37 per cent for those whose mothers received the same diets plus 2 per cent of calcium carbonate.

The best result was obtained on the diets containing 0.5 per cent of added calcium carbonate; only 11 per cent of the 129 young born in first litters to the 15 females on these diets died between birth and weaning. The results with the diets containing 1 per cent of added calcium carbonate were about equal to those obtained with the basal diets alone.

The experiment was continued through a period of eighty days so that the complete study included data from 3 to 4 litters for each female. These complete results likewise indicated a definite deleterious effect from the higher calcium carbonate levels, even though there was a tendency, in second and subsequent litters, toward improvement in reproductive performance among all groups. Females on the diets containing 0 and 0.5 per cent of added calcium carbonate weaned a total of 247 and 274 young out of 313 and 307 births, respectively. Those receiving 1.0 and 2.0 per cent of added cal-

cium carbonate weaned 217 and 154 young out of 290 and 274 births, respectively.

In a second experiment, the effects were determined of additions of iron and phosphorus to the fourth basal diet containing either 0.5 or 2.0 per cent of added calcium carbonate. Ferric citrate was used to increase the iron content of the diet from 35 to 45 parts per million. Sodium dihydrogen phosphate was used to narrow the calcium-phosphorus ratio of the diet from 2.29 to 1.09. A group was included in which both the iron and the phosphorus supplements were combined.

The higher calcium diet with no iron or phosphorus supplement was again inferior to the basal diet with the lower calcium addition, based on the total weight of young weaned and on the weight per litter weaned. Additions of either the iron or the phosphorus or the combination of these eliminated the deleterious effects of the higher calcium diet.

Postmortem examinations of the young mice at weaning revealed the frequent occurrence of pale, speckled livers, enlarged hearts, and small thymus glands; these conditions were noted to be particularly pronounced on the diets with the higher calcium additions. For example, first-litter young whose mothers were receiving a supplement of 0.5 per cent of calcium carbonate had heart weights equal to 959 mg. per 100 g. of body weight, contrasted with heart weights of 2133 mg. per 100 g. of body weight when the diet of the mothers contained 2.0 per cent of added calcium carbonate. The higher calcium supplement reduced thymus weights from 329 to 158 mg. per 100 g. of body weight.

W. A. Greig (*Brit. J. Nutrition* **6,** *280 (1952)*) reported the influence of calcium carbonate supplements to the basal diets on the blood hemoglobin levels. The breeding females receiving the 2.0 per cent calcium carbonate supplement had significantly lower blood hemoglobin levels (11.8 to 12.9 g. per cent) than those receiving no supplement (14.2 to 15.3 g. per cent).

In the young mice the effect of the higher calcium supplement was to reduce the blood hemoglobin from an average of 5.0 to 2.7 g. per cent. The anemia presented the hematologic features of an iron deficiency and was corrected by the supplements of iron but not by phosphorus. In association with the anemia in the young mice, there were fatty deposits in the liver and marked hyperlipemia.

The British workers feel that the reproductive impairment resulting from calcium carbonate additions to the basal diets can be explained on the hypothesis that the calcium in some way interferes with the availability of the iron in the diets. They present a thorough review of the literature in which it is emphasized that there is a distinct division of opinion as to the effects of dietary calcium salts and the calcium-phosphorus ratio on iron absorption by animals.

A number of investigators have demonstrated that calcium additions to the diet reduce iron storage in the liver, reduce blood hemoglobin levels, and decrease iron retention or absorption. On the other hand, the view that low dietary calcium or high dietary phosphates will inhibit iron absorption has also been widely accepted and is based on observations that iron deficiency anemias develop on low calcium-phosphorus ratios unless calcium or some other phosphate-binding ion is also fed.

It is generally believed that iron absorption in animals is governed largely by the concentration of ferritin (a protein and colloidal ferric hydroxide) in the intestinal mucosa, and that absorption occurs only when the iron of ferritin is reduced to a critical point. Recent studies have indicated that iron absorption through this mechanism can be disturbed under certain abnormal conditions such as pyridoxine deficiency (C. J. Gubler, G. E. Cartwright, and M. M. Wintrobe, *J. Biol. Chem.* **178,** *989 (1949)*). An abnormally high absorp-

tion of iron and the deposition of hemosiderin in tissues was observed to be caused by a deficiency of minerals, particularly phosphate (D. M. Hegsted, C. A. Finch, and T. D. Kinney, *J. Exp. Med.* **90,** *147 (1949)*).

The diet of the British workers which produced the best reproductive performance in mice (basal diet plus 0.5 per cent of calcium carbonate) contained about 0.55 per cent of calcium, whereas the diets with the higher calcium supplements contained 0.80 to 1.1 per cent of calcium. In extensive studies by Sherman with rats, somewhat superior results were obtained with similar diets containing 0.80 per cent of calcium than with dietary calcium levels of 0.35 or 0.64 per cent (H. C. Sherman, "Calcium and Phosphorus in Foods and Nutrition," *Columbia University Press, New York (1947)*).

The recent British studies again emphasize that the balance of minerals is of utmost importance in nutrition and that the precise interrelationship of iron, calcium and phosphorus is not yet clear.

A NEW FORM OF VITAMIN D

The first of the D vitamins to be thoroughly investigated was calciferol—vitamin D_2—an entirely artificial product made by exposing ergosterol, a sterol found exclusively in plants, in particular fungi, to the action of ultraviolet light. The photochemical changes occurring under irradiation involve a rearrangement of the molecule, in particular a rupture of one of the rings in the sterol nucleus and the appearance of a system of three conjugated double bonds. The wave lengths of about 230 to 305 millimicrons are found to be most effective, and the series of compounds which appear upon irradiation has been identified and the substances named: lumisterol, tachysterol, calciferol, suprasterols and toxisterols. Vitamin D_2 is characterized by an absorption spectrum presenting maximum absorption of 265 millimicrons. Its activity is 40,000 I. U. per milligram.

Vitamin D_3 is formed in the same way as vitamin D_2, but the precursors differ. In this case, 7-dehydrocholesterol, an animal sterol synthesized in the mammalian body, is the precursor. The absorption spectrum of vitamin D_3 closely resembles that of vitamin D_2. When measured in rats, the antirachitic potency of vitamin D_3 is the same as that of vitamin D_2, although it is considerably greater when measured in chickens.

Aside from these two essential forms of the vitamin, other molecules of related structures have been shown to have some antirachitic potency. Vitamin D_4 is activated 22-dihydrocalciferol; 7-dehydrositosterol and cholesterilene, a product of treatment of cholesterol with fuller's earth, have been shown to be very slightly antirachitic for rats; 7-dehydrocholesterol and irradiated cholesterol, the irradiated product formed by heat treatment of 7-ketocholesteryl acetate, and a product prepared by the treatment of ergosterol with nitrites, have also been claimed to show weak vitamin D potency. C. E. Bills, O. N. Massengale, M. Imboden, and H. Hall (*J. Nutrition* **13,** *435 (1937)*) suggested that the antirachitic activity of fish liver oils was due to more than one product, but this indication was not pursued experimentally. Recent publications by Y. Raoul and his collaborators have now reopened the problem of the multiple nature of vitamin D.

In a series of reports (Y. Raoul *et al.*, *Compt. rend.* **229,** *259 (1949)*; **232,** *1258 (1951)*; **234,** *2493 (1952)*) the French workers described in detail the initial steps in the activation of 2–4, 2′–4′ bicholestadiene, a product of partial oxidation of cholesterol containing two double bonds. An intermediate ("substance P") characterized by

a maximum of absorption at 255 millimicrons can be transformed by chemical treatment into two closely related compounds ("substance A" and "substance B"), both of which show vitamin D potency. More remarkable, the activity of these compounds assayed in rats is 20,000 I. U. per milligram, of the same order of magnitude as that of vitamins D_2 and D_3.

In a more recent report (Y. Raoul *et al.*, *Compt. rend.* **235,** *1439 (1952)*) the Paris workers demonstrated that the new form of the vitamin which they call vitamin D_7, as well as its inactive precursor (substance P), can be isolated from fish liver oils. One method, yielding the active form as a calcium complex, is based on saponification of cod liver oil, followed by a series of ether extractions. A number of adsorptions on alumina columns, followed by elution, concentrations *in vacuo*, and low temperature precipitations are used in the final purification. The precursor is most easily prepared from shark liver oil concentrate, adsorbed on alumina columns and eluted, and freed from vitamin A and preformed vitamin D_7 by repeated carbon tetrachloride extraction. The method involves saponification followed by a number of reprecipitations. Cod liver oil was found to contain appreciable amounts of both vitamin D_7 and its precursor, tuna liver oil to be high in the preformed vitamin, while shark liver oil was especially rich in "precursor" content. Carbon and hydrogen determinations were conducted on the isolated active substance and on the precursor, as well as on the synthetic vitamin D_7 and "substance P" respectively. Agreement for the values found for the natural and the "synthetic" substances was good. The activity of the natural and synthetic vitamins was found to be similar on a weight basis.

These reports make it highly probable that vitamin D_3 is only one of a number of physiologically active vitamins D existing in vertebrates. Elucidation of the structure of the new substance and comparison with the classic irradiation products of ergosterol and 7-dehydrocholesterol should help to determine what part of the vitamin D molecule is indispensable from the point of view of chemical specificity.

GROWTH STIMULATION BY COCONUT MILK

In 1948 it was pointed out (S. M. Caplin and F. C. Steward, *Science* **108,** *655 (1948)*) that rapid and random division of mature cells in higher plants could be induced *in vitro*. In this instance cylinders 1 mm. x 2 mm. and weighing about 4 mg. were punched out of carrot roots so that one end of the phloem cylinder approximated the cambium; these cylinders were incubated in a nutrient medium while the containing vessels were agitated to produce aeration of the plant tissue. It was observed that among certain adjuvants to the medium which exerted an accelerating effect on the proliferation of the phloem cells of the carrot root, coconut milk in the concentration of 15 per cent was particularly effective. This natural plant juice is the fluid endosperm nourishing an immature embryo which later produces a spongy mass of cotyledonary tissue that ultimately fills the central cavity of the fruit. It was concluded that the growth-promoting factor in the coconut milk was not a mineral, thiamine, pyridoxine, or glycine.

In a more recent study (E. M. Shantz and F. C. Steward, *J. Am. Chem. Soc.* **74,** *6133 (1952)*) there is given the result of efforts to fractionate the coconut milk in order to identify the growth-promoting substance contained therein. At least three factors can be recognized as chemical entities, though there may be more. The coconut milk, diluted with an equal volume of

ethyl alcohol, was cleared with mercuric acetate, the mercury removed and the filtrate concentrated to a sludge which was extracted with 90 per cent ethanol. The heavy syrup resulting from the removal of the alcohol showed optimal activity with the carrot slice culture growth test when added to the basal medium at a level of 200 parts per million (p.p.m.). When the foregoing extract was further fractionated by differential solubility in various solvents or by partition chromatography on cellulose, fractions were obtained which were less potent in promoting cell growth than was the whole coconut juice. The full growth response could be elicited, however, by adding the free amino acid-containing fraction of the coconut milk, enzyme-hydrolyzed casein, or pure amino acids. This supplementary action of hydrolyzed casein suggested its use with various fractions of the coconut milk which in themselves were ineffective, and three substances have been isolated which, with hydrolyzed casein, stimulate growth at a rate approaching that induced by whole coconut milk alone.

The concentrated alcoholic extract of the mercury-free precipitate yielded crystals (compound A) which melted at 240.5° C. (uncorrected) containing 14.19 per cent nitrogen and which at a level of 1.0 p.p.m. in the basal medium and with hydrolyzed casein, stimulated the same growth as did the unaltered coconut milk. Crude concentrated alcoholic concentrate (equivalent to 200 gallons of the original coconut milk) was further fractionated with suitable solvents and chromatographed in n-butyl alcohol-acetic acid-water mixture on a powdered cellulose column. The fractions, 300 in number, were characterized by their ultraviolet absorption, ultraviolet fluorescence and reaction with ninhydrin. Strong growth stimulation was evident in the early fractions, and free phenylalanine and tyrosine were identified. Between the phenylalanine and the tyrosine were fractions giving no ninhydrin test.

From these compounds, compound B was isolated in crystalline form; it stimulated growth equal to that of the original coconut milk at a level of 0.25 p.p.m. From the same fractions crystals of compound C were obtained; it contained 7.67 per cent nitrogen and produced growth in a concentration of 2.0 p.p.m. The coconut milk growth factor appears to consist of a number of substances, closely related, but of unknown identity. Coconut milk has been shown to stimulate the growth of fungi, bacteria and orchids as well as of the cells in carrot and beet; in view of the striking potency of this endosperm structure, further investigation of its composition is warranted.

EXPERIMENTAL HYPERLIPEMIA AND HYPERCHOLESTEROLEMIA OF RATS

The study of the mechanism of hyperlipemia and hypercholesterolemia has been advanced by the development of a new experimental method of inducing these disturbances in animals. A. Kellner, J. W. Correll, and A. T. Ladd (*J. Exp. Med.* **93,** *373, 385 (1951)*) produced hyperlipemia and hypercholesterolemia by the intravenous injection of rabbits with Tween 80 and Triton WR-1339, a polymer of para-iso-octyl polyoxyethylene phenol. Other surface-active compounds have been demonstrated to have a similar action. One such compound, cholic acid, has been extensively investigated by M. Friedman and S. O. Byers (*Am. J. Physiol.* **168,** *292 (1952)*). These authors have presented evidence indicating that a rise in plasma cholate level may be responsible for the rise in blood lipids in certain disease states.

The mechanism whereby Triton WR-1339 results in hyperlipemia and hypercholesterolemia has been the subject of a recent report (M. Friedman and S. O. Byers,

J. Exp. Med. **97,** *117* (*1953*)). The methods used in the study of cholesterol metabolism by this laboratory have been reviewed recently in reference to cholesterol metabolism in hyperthyroidism and hypothyroidism (*Nutrition Reviews* **10,** *310* (*1952*)).

The intravenous injection of from 50 to 150 mg. of Triton into rats weighing about 250 g. resulted in a rise of plasma cholesterol and total lipid. These changes occurred promptly. By four hours plasma cholesterol had risen from 55 to 105 mg. per cent. A maximum cholesterol value of 456 mg. per cent was reached in forty-eight hours with a subsequent decline almost to the original value by one hundred and twenty hours. The percentage increase which occurred in plasma total lipid, phospholipid and neutral fat concentrations was even greater than that occurring in plasma cholesterol.

Because of the apparent importance of elevated blood cholate levels in certain hypercholesterolemic states as reported from this laboratory, the plasma cholate level was measured following Triton administration. The plasma level rose from 6 mg. per cent to 21 mg. per cent. Ingestion of cholate did not cause a further elevation of cholesterol after Triton administration. Neither biliary duct ligation nor creation of a biliary fistula significantly modified the hypercholesterolemia which followed Triton injection. It was concluded that the hypercholesterolemia evoked by Triton was not mediated by increased cholate levels. It was postulated that Triton increased the avidity of the plasma for cholate because high levels of cholate persisted in animals with biliary fistulas. Control animals with biliary fistulas developed low plasma cholate levels.

Having eliminated an increase in plasma cholate as the responsible mechanism of Triton-induced hypercholesterolemia, the authors investigated the source of the excess plasma cholesterol. Functional hepatectomy prevented the rise in plasma cholesterol. As an index of hepatic cholesterol production the excretion of cholesterol in the bile was measured. The experimental evidence supporting such an interpretation had been presented previously by S. O. Byers and M. Friedman (*Am. J. Physiol.* **168,** *297* (*1952*)). Following the intravenous injection of a single dose of Triton the biliary cholesterol was 1.85 mg. per twenty-four hours whereas control rats excreted 2.44 mg. per twenty-four hours. Rats receiving repeated doses of Triton excreted 2.09 mg. per twenty-four hours. On the basis of these observations the authors have concluded that the hypercholesterolemia produced by Triton was not caused by an increase in the rate of hepatic synthesis.

Despite the fact that plasma cholesterol and plasma lipids rose as much as ten times the fasting level in Triton-treated rats, no accumulation of sudanophilic material was seen in histologic sections of liver, kidney, adrenal, heart, lung, or intestines. Also, the total cholesterol content of the liver, and the carcass minus head and liver, was the same in control and Triton-treated rats. The excess cholesterol could not have been due to a loss in the preformed cholesterol of either liver or carcass.

The explanation offered for the results obtained is that the accumulation of cholesterol after Triton injection is due to impaired removal of cholesterol from the blood. The removal of cholesterol from the blood by the gastrointestinal tract was considered. It was found that the excretion of fecal cholesterol and total digitonin precipitable sterols was the same in normal and Triton-treated rats. Studies of cholate excretion in Triton-treated rats also suggested that there was no impairment of conversion of cholesterol to cholate. The authors have hypothesized that the cholesterol metabolism of the tissues is normal after Triton administration, but there is an alteration of the physical state of cholesterol in the plasma which made it unavailable for metabolism. An attempt was made to show that the blood itself rather than the tissues was abnormal after Triton injection. Exchange

transfusion between Triton-treated rats did not prevent the rise of plasma cholesterol. When the blood of Triton-treated rats was replaced by normal blood, hypercholesterolemia was greatly reduced. Normal rats receiving blood from rats treated with Triton six hours before bleeding developed hypercholesterolemia. Further evidence of an abnormality of serum lipid following Triton administration was obtained by ultracentrifugal analysis. The disappearance of normal serum lipoproteins and the appearance of lipoproteins of high flotation rates were found.

Friedman and Byers have made a significant contribution to the study of cholesterol metabolism. Although the increase in plasma lipids following Triton administration was greater than plasma cholesterol, the metabolism of the lipids other than cholesterol merits more investigation. The assumption that a similar mechanism exists for the accumulation of plasma lipids requires verification. The physical-chemical nature of plasma lipid transport gains added importance from these studies. The possible importance of cholate or other surface-active substances in producing hypercholesterolemia in human disease has been reviewed recently by Byers, Friedman, and R. H. Rosenman (*Metabolism* **1**, *479 (1952)*). This should be a profitable investigative approach in the study of human disease.

STUDIES ON THE B_{12} VITAMINS

Intensive investigations of the B_{12} vitamins have been carried on by a group of British scientists since 1949 at the Glaxo Laboratories in Middlesex, England. Attempts to label the vitamin with radioactive isotopes to facilitate metabolism studies were begun soon after the isolation of crystalline vitamin B_{12}.

Direct irradiation of vitamin B_{12} in the atomic pile was undertaken since direct chemical exchange with radioactive cobalt compounds and radioactive phosphate introduced no significant radioactivity. E. L. Smith (*Biochem. J.* **52**, *384 (1952)*) reported that irradiation with thermal neutrons induced some radioactivity in the molecule without causing excess decomposition. After four weeks exposure to a low neutron flux, anhydrous crystalline vitamin B_{12}, sealed *in vacuo*, had accumulated some radioactivity. A small portion of the induced activity remained associated with the vitamin B_{12} after thorough purification by solvent extraction, recrystallization and chromatography. Specific radioactivities were too low for the products to be of value in biologic experiments.

The second experiment used a larger amount of microcrystalline material, not sealed *in vacuo*, and produced a lower specific radioactivity. Purification was done rapidly to detect possible activation of the phosphorus, and it was found that phosphorus as well as cobalt in the vitamin B_{12} had become radioactive.

Further attempts to obtain radioactive vitamin B_{12} involved biosynthesis. By using *Streptomyces griseus* growing in a semi-synthetic medium containing radiocobalt, E. L. Smith, D. J. D. Hockenhull, and A. R. J. Quilter (*Biochem. J.* **52**, *387 (1952)*) were able to extract from the fermentation liquors vitamin B_{12} with a specific activity of 120 microcuries per milligram, considerably higher than previously recorded.

Preparation of vitamin B_{12} with radioactive phosphorus was undertaken since phosphorus would be safer for projected human metabolism tests than the penetrating radiation of Co^{60}-vitamin B_{12}. P^{32} was incorporated into vitamin B_{12}, but at low activity. Less than one part in ten thousand of the P^{32} added was present in the vitamin B_{12} produced by the fermentation. Competing metabolic requirements for phosphorus resulted in a low efficiency

of conversion and a specific activity of only 0.35 microcuries per milligram.

E. L. Smith, S. Ball, and D. M. Ireland (*Biochem. J.* **52,** *395* (*1952*)) isolated three red crystalline antipernicious anemia factors from *Streptomyces griseus* fermentation liquors. The yield of the new factor, vitamin B_{12c}, was very small, but was much enhanced by treating vitamin B_{12b} by nitrous acid. Nitrous acid appeared to have no effect on vitamin B_{12} itself, but it readily converted vitamin B_{12b} into vitamin B_{12c}. B_{12c} could readily be converted into a basic factor called vitamin B_{12d}. This also occurred on catalytic hydrogenation and from mild hydrolysis. Evidently the molecule of vitamin B_{12c} incorporated one nitrite group, probably linked directly to the cobalt atom. Vitamin B_{12c} in fermentation liquor occurred in concentrates not treated with nitrous acid. It was not necessarily synthesized directly by the mold. A trace of nitrite in the fermentation liquor could convert part of the vitamin B_{12b} present into vitamin B_{12c}. Removal of this nitrite group by treatment with sulfamic acid or distillation of an acidified solution yielded vitamin B_{12b} again.

This work was done before anything was known of the constitution of vitamin B_{12}. Nitrous acid was believed to cause some chemical change in vitamin B_{12b} besides being added on reversibly. In that event, the denitrosation product, which was called vitamin B_{12d}, would differ from vitamin B_{12b}. The absorption spectrums of these two compounds were identical, including the shift at alkaline pH values already recorded for vitamin B_{12b} (J. A. Brockman *et al., American Chemical Society, 117th meeting, p. 11A* (*1950*)).

Careful studies of partition chromatograms resulted in mixtures of vitamins B_{12b} and "B_{12d}" that could not be separated. Their identity was confirmed by tracer technics. Partition chromatograms were prepared of mixtures of vitamin B_{12b} and radioactive vitamin B_{12d}. Upper and lower sections of the single red band resulting gave materials of identical specific activity.

The basicity of vitamin B_{12b} was revealed by its more complete extraction from alkaline than neutral or acidic solutions by organic solvent. The main ultraviolet absorption band of vitamin B_{12c} in neutral aqueous solution occurred at 352 millimicrons, that of rigorously purified vitamin B_{12b} was a narrower band at 351 millimicrons. Polarographic behavior of vitamins B_{12b} and B_{12c} indicated that the cobalt atom is bound in a complex and is not ionizable. Electric conductivity studies indicated that vitamin B_{12c} is weakly ionized since its conductivity approximates that of vitamin B_{12}. Vitamin B_{12c} is diamagnetic. Vitamin B_{12c} rotates in the opposite direction from vitamins B_{12} and B_{12b}. Vitamins B_{12b} and B_{12c} are just as effective as vitamin B_{12} itself against pernicious anemia.

Reactions of the B_{12} vitamins with sulfur dioxide, cyanide and other reagents studied by Smith *et al.* (*Biochem. J.* **52,** *395* (*1952*), *loc. cit.*) indicate that they form adducts readily in a manner similar to heme pigments.

The structures and stabilities of these cobalamines have been studied, mainly by tracer technics and by measurements of absorption spectrums and partition coefficients. These studies by Smith and his co-workers form an interesting and informative resumé of vitamin B_{12} chemistry.

CORTISONE AND PROTEIN SYNTHESIS

The perennial objective of studies in intermediary metabolism is to secure more information regarding the mechanism of cellular intervention in chemical reactions. Progress is measured by the invention of new philosophic or operational approaches

toward this objective. One of these is the procedure of D. B. Sprinson and D. Rittenberg (*J. Biol. Chem.* **180,** *715 (1949)*), whereby the rate of protein synthesis can be calculated from the amount of urinary nitrogen after the feeding of a tracer dose of labeled (N^{15}) glycine. Another fruitful circumstance is the now well-known fact that the excretion of nitrogen in either fed or fasting experimental animals is increased after the injection of adrenal cortical steroids having oxygen substitution groups at the 11-position. A recent investigation (I. Clark, *Ibid.* **200,** *69 (1953)*) has employed the foregoing devices to elicit further information regarding protein metabolism.

It is immediately obvious that the augmented loss of nitrogen in the urine following the administration of cortisone may arise from either an inhibition of protein synthesis or an exaggeration of protein breakdown. The experimental approach was through the administration of N^{15}-labeled glycine to both normal and adrenalectomized rats, maintained on a slightly smaller than ad libitum food allowance, and receiving a 1 per cent sodium chloride solution instead of drinking water. Part of each group was treated with 3 mg. of cortisone acetate administered subcutaneously daily for five days. On the fourth day the isotopically labeled amino acid was given by stomach tube and the urine was collected for two days thereafter; total nitrogen, urea nitrogen, ammonia nitrogen and N^{15} were estimated quantitatively.

That there was an interference with the metabolism of protein after administration of cortisone was plain at the outset inasmuch as the hormone-treated rats lost weight despite the same food intake as that of the controls. Again, the comparative data show a more pronounced loss of nitrogen in the urine of those animals (either intact or adrenalectomized) which had received cortisone. Furthermore, the adrenalectomized controls showed a somewhat smaller loss of both total and urea nitrogen than did the intact controls, indicating a retention of nitrogen in the absence of available cortical steroids. A study of the data on excretion of isotopic nitrogen showed that more N^{15} was excreted by the hormone-injected rats than by either of the control groups. When the rate of protein synthesis was calculated for all of the groups it appeared that the administration of cortisone results in an over-all decrease in both the intact controls and the adrenalectomized animals; in contrast there was a slight, statistically insignificant increase in the rate of protein synthesis in the adrenalectomized controls over that in the intact controls. The foregoing data suggest that, physiologically, the loss of nitrogen in the urine after administration of cortisone is brought about by the interference with protein synthesis from possible nonprotein dietary precursors.

A second group of rats was utilized for tissue studies; they were given cortisone for ten days and the labeled glycine on the last two days. Urine was collected on the tenth and eleventh days and the animals were killed. Serum, the defatted carcass, liver, and the alcohol-ether extract were analyzed for nitrogen, creatinine (extract only) and N^{15}. Again, loss of body weight under the uniformly restricted feeding was greater when cortisone was given, and the data show a marked increase in loss of total and urea nitrogen and of N^{15}, suggesting that cortisone impairs the utilization of dietary protein.

Both the total nitrogen of the tissue and the nonprotein nitrogen of the extract were lower in the cortisone-treated animals and the ratio of the contained N^{15} to fed N^{15} was decreased in tissues and extracts after cortisone was injected, suggesting, again, a lowered efficiency of protein synthesis after the hormone was administered. On the other hand, the livers of the cortisone-treated rats were larger and contained more protein nitrogen and more N^{15}, all of which points to an enhanced rate of protein synthesis in this organ under the influence

of cortisone, a conclusion supported by the higher level of serum proteins (formed in the liver) and the somewhat greater N^{15} concentration in the serum.

Thus the influence of the steroid hormone on the liver is opposite to that on tissue proteins. One suggestion is that, in view of the delay of protein synthesis in the tissues, the consequent accumulation of nonprotein dietary precursors forces the liver to greater detoxication activity. Thus the analytic evidence of heightened hepatic activity in these experiments represents a compensatory reaction by the liver. However, the comparative mass of hepatic tissue is so small that the over-all picture in the body is one of inhibition of protein synthesis by cortisone.

AN EFFECT OF THE ADRENAL MEDULLA ON WATER DIURESIS

The diuresis induced by the administration of water is diminished or delayed in experimental animals or patients with adrenal insufficiency. This defect is modified by administration of cortical hormones and has been considered to be the result of decreased adrenocortical activity (F. J. Robinson, M. H. Power, and E. J. Kepler, *Proc. Staff Meetings Mayo Clinic* **16,** *577 (1941)*; R. Gaunt, J. H. Birnie, and W. J. Eversole, *Physiol. Rev.* **29,** *281 (1949)*). Some revision of this concept may be indicated in view of the recently described experiments of D. Dexter and H. B. Stoner (*J. Physiol.* **118,** *486 (1952)*), which demonstrate that the adrenal medulla has an important role in water diuresis in rats.

The animals used in these experiments were immature male albino rats. The medulla of both adrenal glands was removed by a surgical procedure, leaving the capsule together with an adhering layer of glomerulosa cells of the cortex. The animals were provided a solution of 1 per cent sodium chloride for drinking purposes for the first postoperative week. Tests of water diuresis were not undertaken until a period of at least thirty-two days had elapsed following operation. Each rat was used for a single diuresis experiment. Most animals were killed after the experimental procedure and histologic examination for medullary tissue in the adrenal was made.

Significant in an interpretation of the experiments performed is an assessment of the results of the surgical procedure. Following medullectomy there was formation of scar tissue in the region of the adrenal medulla. In contrast, there was regeneration of the cortex so that the adrenal weight on the thirty-second day was about 80 per cent of that of control animals of similar body weight. At this time the histologic appearance of the regenerated adrenal cortex was normal and the content of ascorbic acid was about 80 per cent of that of control animals. It was demonstrated that the regenerated adrenal cortex of the medullectomized animals possessed functional capacities comparable to those of normal rats. Tests of adrenocortical function included the reduction in ascorbic acid content of the gland following injection of adrenocorticotropic hormone, increase in weight of the adrenal cortex with prolonged exposure of the animals to cold, decrease in blood sugar levels with fasting, and determination of the total work capacity of the gastrocnemius muscle. The data presented give substantial proof to the contention of the authors that the medullectomized rats used in their experiments retained adequate and effective adrenocortical function. The animals were otherwise in good health, consuming food at the same rate and with growth proceeding as in normal or control rats.

Water diuresis was undertaken under a standard procedure. The animals were given 5.0 ml. of water per 100 g. of body weight

by stomach tube late in the day prior to the experiment and then allowed free access to water overnight. In the experiment, 5.0 ml. of water per 100 g. of body weight was administered by stomach tube at intervals of one hour for three hours. The quantity of urine excreted per hour was then determined. It was found that medullectomized rats excreted only half as much water as control animals of the same weight during the first two hours of such an experiment. The differences were determined to be statistically highly significant. However, the excess water was not indefinitely retained in the experimental animals. Total urinary excretion after twelve hours was the same in groups of medullectomized and control animals. The effect of removal of the adrenal medulla was, therefore, one of delay in excretion of the administered water. This effect occurred in rats tested relatively late after medullectomy, fifty-six to ninety days, as well as earlier, thirty-six to thirty-eight days. Moreover, the diuretic response was similar in medullectomized animals with adrenocortical hypertrophy induced by prolonged exposure to low temperatures. The quantity of water administered did not produce signs of water intoxication in any of the normal animals of the control group, but a fall in body temperature indicating intoxication did occur on occasion in medullectomized rats. Determination of rates of water absorption demonstrated no differences between the two groups. The relatively large quantities of water utilized in producing diuresis in these experiments produced a significant increase in blood sugar and a decrease of about 25 per cent in the adrenal content of ascorbic acid in normal rats. Neither effect occurred in the medullectomized animals, however.

The results of these experiments were compared with similar tests of water diuresis in totally adrenalectomized rats maintained on sodium chloride. These latter animals showed an extremely poor response to the three doses of water and all of them had manifestations of water intoxication. Parenteral administration of adrenal hormones modified the response to water in these adrenalectomized animals. Cortisone demonstrated a striking effect, preventing water intoxication and increasing diuresis to levels similar to those of normal animals. Also, D,L-nor-epinephrine reduced water intoxication and caused a marked initial increase in the rate of diuresis persisting for no longer than three hours, following which the rate was comparable to that of untreated adrenalectomized rats. The tolerance to water was similarly increased by administration of L-epinephrine. However, this hormone was without effect on the first two hours of diuresis, after which a marked and sustained increase in urinary excretion occurred.

These three hormones were administered similarly to medullectomized animals. The effects were of lesser magnitude, but similar to those observed in the adrenalectomized rats, with cortisone demonstrating greatest activity.

These observations show that the diuretic response to water is modified in rats from which the adrenal medulla has been removed. However, the defect is not so great as in the totally adrenalectomized animal. Moreover, the administration of cortisone effects restoration of normal diuretic response in both medullectomized and adrenalectomized animals, while the medullary hormones, D,L-nor-epinephrine and L-epinephrine, bring about only partial restoration of this function. Although these observations point to a greater role of the adrenal cortex in control of water diuresis in the normal animal, they give strong indication that the medulla is also concerned in this process.

The authors consider mechanisms through which the medulla might influence the diuretic response. Certain of the findings are compatible with a role of medullary stimulus of the pituitary, causing production of adrenocorticotropic hormone with conse-

quent release of cortisone by the adrenal cortex. The delay in response of the medullectomized animal to epinephrine would indicate such an indirect mechanism. However, other mechanisms must be involved, since adrenalectomized animals respond similarly to L-epinephrine. In addition, the prompt response of all animals to norepinephrine would seem to indicate a direct effect of that hormone on the kidney.

THIAMINE-SPARING EFFECT OF FAT

It has been known for a long time that a diet rich in carbohydrate brings on the symptoms of thiamine deficiency more rapidly than a diet rich in fat. This has been termed the "sparing action of fat on vitamin B_1" (H. M. Evans and S. Lepkovsky, *J. Biol. Chem.* **83,** *269 (1929)*). H. G. K. Westenbrink (*Acta brev. neerland.* **3,** *95 (1933)*; *Arch. Neerl. de Physiol.* **19,** *94 (1934)*), who gave an early detailed description of this phenomenon, observed that pigeons fed a high carbohydrate, fat-free diet, showed avian polyneuritis in nineteen days while a similar group fed a high fat, carbohydrate-free ration showed the same symptoms only after twenty-seven days on the deficient regimen.

The "thiamine-sparing effect of fat" was confirmed by many workers, in particular D. Melnick and H. Field (*J. Nutrition* **17,** *223 (1939)*) and A. Arnold and C. A. Elvehjem (*Am. J. Physiol.* **126,** *289 (1939)*). The latter workers showed that the thiamine requirement of rats could be decreased by two-thirds when the fat content of the diet was raised from 0 to 56.6 per cent. C. P. Richter, L. E. Holt, B. Barelare, and C. D. Hawkes (*Ibid.* **124,** *596 (1938)*) observed that rats made deficient in thiamine and put on a self-selection diet chose a high fat regimen and displayed a marked aversion to carbohydrate and proteins. H. M. Evans, S. Lepkovsky, and E. A. Murphy (*J. Biol. Chem.* **107,** *429 (1934)*) compared various fats from the point of view of their thiamine-sparing action and found that this sparing effect increased with the length of the fatty acid carbon chain, the maximum effect being reached with 8 carbon atoms in the chain (caprylic acid).

If the existence of a sparing action of fat on thiamine was thus clearly established, its significance remained in doubt. It could mean that an increased carbohydrate ingestion entails a greater loss of thiamine from the tissues. It could also mean that a high intake of carbohydrate causes symptoms of thiamine deficiency to appear at a concentration of this vitamin in the tissues at which these symptoms would not appear if a high fat diet was consumed. This question has been resolved by M. Gruber, one of Westenbrink's collaborators.

In one experimental series (*Nature* **166,** *78 (1950)*) Gruber placed three groups of 10 male pigeons on the following thiamine-deficient diets which were force-fed directly into their stomachs: Group A: 2 g. of casein, 18 g. of sucrose; Group B: 2 g. of casein, 6 g. of sucrose; Group C: 2 g. of casein, 8 g. of peanut oil. All three groups also received supplements of minerals and all necessary vitamins other than thiamine. After thirteen days, the animals were killed. The livers, hearts and cerebrums were minced and the thiamine pyrophosphate content of these tissues was determined. It was found that for all three tissues diet A (high carbohydrate diet) caused a significantly greater depletion than diet B (moderate carbohydrate), which in turn gave rise to significantly lower thiamine pyrophosphate levels than diet C (high fat). For example, the liver cocarboxylase levels on the high carbohydrate diet were only one third of those on the moderate carbohydrate

intake and one fifth of those of the pigeons fed the high fat regimen. Differences in intestinal synthesis of the vitamin or in protein content of the tissues were ruled out, so that the only remaining alternative is that increased carbohydrate intake leads to increased disappearance of the thiamine pyrophosphate, a coenzyme in carbohydrate metabolism.

It still remained possible, however, that besides this diminished rate of disappearance on a high fat diet, the second possibility, survival on lower tissue thiamine level, took place simultaneously. In a second experimental series, M. Gruber (*Biochim. et biophys. acta* **9,** *333* (*1952*)) determined the thiamine pyrophosphate tissue levels at the time of death from the deficiency in two groups of 35 and 36 pigeons force-fed respectively the high carbohydrate ration (diet A) and the high fat ration (diet C). The following tissues were examined: liver, heart, breast muscle, cerebrum. It was found that on the high carbohydrate diet liver and heart thiamine pyrophosphate levels were significantly lower, and breast muscle and cerebrum levels significantly higher, than on the high fat diet. The differences, in all cases, were less than 40 per cent of the greater value. It is therefore not impossible that failure of two different organs are implicated when the pigeons die after having been fed the two different diets. It is more probable, however, that the critical center is the same in both cases and that whether on a high fat or on a high carbohydrate diet, the lethal level is the same. At any rate, thiamine pyrophosphate levels on both diets at death were similar enough to rule out any "toxic" effect of carbohydrate on thiamine-deficient animals.

It therefore appears established from Gruber's results that the "sparing action of fat" really means decreased destruction of the vitamin on a high fat diet and additional differences in survival on different diets due to different distributions of the phosphorylated vitamin probably play only a minor role.

THE ORIGIN OF URINARY CHOLESTEROL

That a small amount of cholesterol is a constant constituent of normal human urine was claimed by H. Pribram (*Biochem. Z.* **1,** *413* (*1906*)). E. Gérard (*Compt. rend. Soc. de biol.* **70,** *998* (*1911*)) obtained 11 mg. of "impure cholesterol" from 70.9 liters of normal urine. Gross (*Chem. Zentralbl.* **4,** *220* (*1922*)) and A. M. Gaál (*Z. f. klin. Med.* **113,** *349* (*1930*)) later denied that cholesterol was normally found in the urine, but their claims ran contrary to the evidence of a number of other workers, in particular J. A. Gardner and H. Gainsborough (*Biochem. J.* **19,** *667* (*1925*)). A. Butenandt and H. Dannenbaum (*Z. f. physiol. chem.* **248,** *151* (*1937*)) treated 450 liters of normal urine by acid hydrolysis, extraction and digitonide precipitation. From the weight of chemically pure cholesterol esters thus obtained, they estimated that urine normally contains 0.6 mg. of cholesterol per liter. An excretion level of the order of 1 mg. per day was later found by other workers.

While the presence of a minute amount of cholesterol in normal urine seemed thus established, a number of questions remained unanswered. In particular, the state of cholesterol in urine and its origin were not known. Both points have been clarified by a recent report of F. Kayser and R. Balat (*Bull. Soc. chim. biol.* **34,** *806* (*1952*)).

In a series of experiments Kayser and Balat attempted to see whether human urinary cholesterol was in solution, as claimed by E. Bloch and H. Sobotka (*J. Biol. Chem.* **124,** *567* (*1939*)), or whether it

always accompanied the cellular debris in urinary sediments, as claimed by Y. Bouvrain, R. Tricot, and P. Meudic (*Presse méd.* **59,** *1437 (1951)*). Results of centrifuge studies indicated that, while a variable proportion of urinary cholesterol could be precipitated with the sediment, about two-thirds was actually in solution. This fraction could be "ultrafiltered" through collodion membranes.

Bloch and Sobotka had further theorized that bile salts must be responsible for the excretion of cholesterol by the kidneys and for its solubility in urine. Kayser and Balat undertook to test this hypothesis. They also attempted to increase experimentally the level of urinary cholesterol. Increasing cholesteroluria, they reasoned, might be of value in the treatment of hypercholesteremia. Addition to blood of salicylate, benzoate, para-aminobenzoate, para-aminosalicylate, oleate, octanol, teepol, bile, cholate and urobilin was made to see if blood cholesterol could thus be made dialyzable through collodion. Results were uniformly negative. Next, bile and its derivatives, sulfamides, and hyaluronidase were injected into guinea pigs to see whether the urinary excretion of cholesterol could be increased. Again the findings were negative. The French workers concluded it was unlikely that bile salts fulfilled the role ascribed to them by Bloch and Sobotka.

Pursuing further the problem of the origin of urinary cholesterol, Kayser and Balat compared the physical state of cholesterol in normal urine and in proteinuric urine from patients with nephroses and with mumps. They found that in the pathologic urines, the cholesterol/albumin ratio was approximately constant for a given individual; none of the cholesterol could be filtered through collodion membranes. These facts and the results of ultracentrifuge studies led to the conclusion that in disease, cholesterol was excreted as a constituent of escaping blood lipoproteins. By contrast, the ultrafiltrable cholesterol fraction of normal urine was associated with neither proteins nor polypeptides. Furthermore, in the case of normal individuals, if the urine was collected by catheter from the bladder, the cholesterol content was reduced by half compared with previous levels. This could mean that normal urinary cholesterol might be secreted with the mucus of the urogenital mucosa, along the ureters, the bladder and the urethra. To confirm this hypothesis, Kayser and Balat determined the basal cholesterol excretion of a group of dogs, then placed catheters in the proximal section of their ureters as near the kidneys as possible so as to by-pass the urogenital tract. The urinary cholesterol excretion, normally of the order of 1.8 mg. per liter, fell to undetectable levels. Kayser and Balat concluded that the origin of urinary cholesterol in the normal individual is extrarenal. An interesting problem of physiology appears to have been settled by these experiments.

NOTES

Letter to the Editor

Dear Sir:

The reviewer of our paper "The Food and Nutritional Situation in Berlin during the Blockade and After" in your January number (*Nutrition Reviews* **11,** *5 (1953)*) has failed to indicate adequately the main contents, which in our opinion is one of the purposes of a review. We refrain from making any comment on the review itself, but would like to point out to readers that the journal in which our paper appeared is not generally available. We invite readers who are interested to apply to one of us for reprints. A shortened version of the paper will be found in the U. S. Public Health Service's *Pub. Health Reports* **67,** *622 (1952)*.

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"Let's Teach Better Nutrition"

The slidefilm "Let's Teach Better Nutrition" is now available in three forms; as a filmstrip keyed to a 33⅓ RPM transcription, or a set of three 78 RPM standard phonograph records, or as individual slides. Distribution is through the Department of Nutrition, Harvard School of Public Health, 695 Huntington Avenue, Boston 15, Mass.

Obesity and the Work of the Heart

The statement is commonly made that the heart of an obese patient is overworked because of the extra burden of fat. That more energy must be expended by an obese individual doing a particular piece of work is quite clear. However, whether in the basal state more cardiac work must be done to supply the increased fat tissue with oxygen is not known. To state it in another way, is the cardiac output increased in proportion to the increase in oxygen consumption required by the metabolically active and respiring fat in an obese person? Measurements designed to provide an answer to this question are presented by H. L. Taylor, J. Brozek, and A. Keys (*J. Clin. Invest.* **31,** *976 (1952)*).

Anthropometric measurements and determinations of cardiac output and related functions were made in 34 male university students, ranging in age between 18 and 31, and varying in build from obese to lean. The measurements were made while the individuals were fasting. No attempt will be made in this note to present all of the correlations and lacks thereof between the various measurements made. In general, however, it was noted that the cardiac output did not increase in proportion to the degree of obesity. Since fat respires and therefore requires oxygen, one would expect to find a better removal of oxygen from the blood by the more obese individuals if the cardiac output does not rise. An increased arteriovenous oxygen difference was found. In fact, good correlation was observed between the arteriovenous oxygen difference and the degree of obesity. The greatest cardiac outputs were found in the more muscular individuals with little fat. Increase of fat in the body produced less increase in cardiac output than an increase in lean body mass.

As Taylor and his co-workers point out, the increase in arteriovenous oxygen difference with increasing obesity may have a protective effect since the "efficiency" of oxygen removal from the blood is improved, thus requiring less cardiac work than would be needed were the cardiac output increased in proportion to the oxygen consumption.

Recent Nutrition Books

Cooperative Nutritional Status Studies in the Northeast Region. III. Dietary Methodology Studies. Charlotte M. Young, Faith W. Chalmers, Helen N. Church, Mary M. Clayton, Lorraine O. Gates, Gladys C. Hagan, Betty F. Steele, Ruth E. Tucker, Anne W. Wertz, and Walter D. Foster. University of Massachusetts Agricultural Experiment Station, Bulletin No. 469, Amherst, Mass., August 1952. Northeast Regional Publication No. 10.

Food for Life. Edited by Ralph W. Gerard. University of Chicago Press, Chicago 37, Illinois. Pp. 304. Price: $4.75.

Annotated Bibliography of Vitamin E, Volume II, 1950 and 1951. Compiled by P. L. Harris and W. F. Kujawski. Distillation Products Industries, Rochester, N. Y., 1952. Pp. 106. Price: $2.00. Distributed by The National Vitamin Foundation, Inc., 150 Broadway, New York 7, N. Y.

Handbook of Diet Therapy. Written and compiled by Dorothea Turner for the American Dietetic Association. Revised edition. University of Chicago Press, Chicago, 1952. Pp. 133. Price: $3.50.

Family Food Consumption in Three Types of Farming Areas of the South. II. An Analysis of Weekly Food Records, Late Winter and Early Spring, 1948. Ada M. Moser, Willamay T. Dean, Beulah Gillaspie, Dorothy Dickins, Josephine Staab, Esther F. Phipard, and R. L. Anderson. Southern Cooperative Series Bulletin 20, November 1951.

The State of Food and Agriculture: Review and Outlook, 1952. Food and Agriculture Organization, Rome, Italy, 1952. Pp. 126. Price: $1.00. Obtainable from the International Documents Service, Columbia University Press, 2960 Broadway, New York 27, N. Y.

Chart—*Nutrition Education Resources for Schools.* Helen S. Lockhart, Department of Nutrition, Harvard University School of Public Health, Boston, Mass., 1952. Size 8½″ x 11″, 25¢; wall size 50¢. Distributed by The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y.

Townsman's Food. Magnus Pyke. Turnstile Press, 10 Great Turnstile, London, W. C. 1, England. Price: 15 shillings (postage 6 pence).

Kwashiorkor in Africa. J. F. Brock and M. Autret. World Health Organization, Palais des Nations, Geneva, Switzerland, 1952. Pp. 77. Price: 5 shillings; $1.00; Swiss francs 4.

Sanitary Milk Control and its Relation to the Sanitary, Nutritive, and Other Qualities of Milk. A. C. Dahlberg, H. S. Adams, and M. E. Held. A Research Study sponsored and supervised by The Committee on Milk Production, Distribution, and Quality of the Agricultural Board and the Food and Nutrition Board, National Research Council. Publication 250, National Academy of Sciences—National Research Council, Washington, D. C., 1953. Pp. 174.

Origins of American Scientists. Robert H. Knapp and H. B. Goodrich. University of Chicago Press, Chicago 37, Ill. Pp. 450. Price: $4.75.

Glycerol. ACS Monograph No. 117. Edited by Carl S. Miner and N. N. Dalton. Reinhold Publishing Corporation, 330 West 42d Street, New York 36, N. Y. Pp. 389. Price: $12.00.

Dietetic Canned Foods. Fruits and Vegetables: Sodium and Proximate Composition. Edited by Franklin C. Bing. National Canners Association, Washington, D. C. Pp. 62.